Moss tasiRNAs Make the Auxin Network Robust

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The *TAS3* tasiRNA pathway has been coopted to regulate diverse developmental processes in plants. In this issue of *Developmental Cell*, Plavskin et al. (2016) explore the role of the pathway in the moss *Physcomitrella patens*. Their results suggest that frequent cooption may be related to unique qualities of tasiRNA-mediated regulation.

The appearance of novel structures during evolution often involves the cooption of preexisting gene regulatory networks (GRNs) (Erwin and Davidson, 2009). Although there are many examples of GRN cooption, the properties that make a particular network susceptible to cooption are poorly understood. In flowering plants, the small RNA *TAS3* tasiRNA pathway regulates diverse developmental processes, suggesting that the pathway has been recruited multiple times during evolution. In this issue of *Developmental Cell*, Plavskin et al. (2016) explore the role of the *TAS3* tasiRNA pathway in the moss *Physcomitrella patens*. Their results strongly suggest that the unique qualities of tasiRNA regulation contribute to the frequent cooption of the auxin GRN.

The plant hormone auxin has been implicated in a wide variety of developmental processes (Wang and Estelle, 2014). The auxin signaling pathway is conserved in all land plants and appears to have been repurposed multiple times during plant evolution to support the development of key innovations such as a vascular system and meristems, as well as roots, leaves, and floral organs (Bennett, 2015). Auxin acts by promoting the degradation of transcriptional repressors called Aux/IAA proteins via an E3 ligase called SCF^{TIR1} (Wang and Estelle, 2014). The Aux/IAAs repress auxin-regulated transcription by interacting with members of a family of DNA-binding transcription factors called ARFs (auxin response factors). Some ARFs have been characterized as transcriptional activators, while others, including *Arabidopsis thaliana* ARF3 and ARF4, are described as repressors (Guilfoyle and Hagen, 2007). ARF3 and ARF4 are members of the B clade of ARF proteins that includes PpARFb1, PpARFb2, PpARFb3, and PpARFb4 from *Physcomitrella* (Plavskin and Timmermans, 2012). The auxin GRN is regulated in a number of ways, but one of the most important is through the action of small RNAs. In flowering plants such as *Arabidopsis*, the *TIR1* gene, encoding the auxin co-receptor F-box protein TIR1, as well as several *ARF* genes, is regulated by miRNAs (Yamamuro et al., 2016). In addition, clade B ARFs are regulated by the *TAS3 trans*-acting short interfering pathway (tasiRNA) (Plavskin and Timmermans, 2012). *TAS3* tasiRNAs are synthesized by a pathway that begins with the *mir390*-dependent cleavage of noncoding *TAS3* RNAs. From these RNAs, RNA-DEPENDENT RNA POLYMERASE6 (RDR6) and SUPPRESSOR OF GENE SILENCING3 (SGS3) make long double-stranded RNAs, which are then converted to 21-nt tasiRNAs by DICER-LIKE4 (DCL4). Some of these tasiRNAs then regulate expression of the *ARF3* and *ARF4* genes. Within flowering plants, the pathway regulates a number of developmental processes, in each case by affecting clade B *ARF* expression. Although each of the small RNAs is conserved in some plant lineages, only the *TAS3* tasiRNA pathway is conserved in all land plants including *Physcomitrella*.

The *Physcomitrella* life cycle begins with germination of a haploid spore and the formation of a filament of cells called a protonema (Prigge and Bezanilla, 2010). Initially, the protonema develops as a chlorophyll-rich chloronema. In the right conditions, the dividing chloronemal cells at the tip of the filament may transition to a more elongated cell called a caulonemal cell. Furthermore, caulonema filaments produce the leafy gametophores that ultimately produce the gametes. Previous studies have shown that the chloronema-to-caulonema transition is dependent on auxin and that the core auxin signaling pathway is conserved in *Physcomitrella* (Plavskin and Timmermans, 2012; Prigge and Bezanilla, 2010; Prigge et al., 2010). The *PpARFb* genes are regulated by both *TAS3* tasiRNAs and another small RNA called miR1219. To explore the role of *TAS3* tasiRNAs in auxin signaling, Plavskin et al. (2016) disrupted the single *PpSGS3* gene, thus eliminating tasiRNAs. The mutant plants exhibited a number of growth defects, including a decrease in chloronemal cell size and a reduction in the number of leafy gametophores. However, the most dramatic defect was the nearly complete absence of caulonemal filaments. Further characterization of the mutant showed that this phenotype is associated with an increase in the level of *PpARFb1*, *PpARFb2*, and *PpARFb4* transcripts. The authors confirmed that misregulation of these three genes is responsible for the *Ppsgs3* phenotype by altering the tasiARF target site in *PpARFb4* (*PpARF4b-GUS-t**) and introducing this construct into wild-type moss. The resulting line displayed a reduction in caulonemal filaments but was not as severely affected as the *Ppsgs3* mutant, suggesting that altered regulation of the other *PpARFb* genes is required to recapitulate the mutant phenotype. Consistent with this, when the miR1219 site was also mutated (*PpARF4b-GUS-m*t**), the plants closely resembled the mutant.

To learn more about the role of the tasiARFs in moss development, the authors compared the expression of *PpARFb4-GUS* with small-RNA-resistant forms of the gene. In wild-type plants, the expression of *PpARFb4-GUS* was restricted to the tip cells of some, but not all, protonema. Importantly, the distribution of

protonema expressing the gene appeared to be random. Mutation of either the tasiARF or mir1219 sites in *PpARFb4-GUS* increased both the number of protonema that expressed the gene and the number of expressing cells at the tip of the filament. When both sites were mutated, the reporter was expressed in nearly every protonema, with a further expansion of the expression zone away from the tip. Because the PpARFb proteins are thought to repress auxin signaling, these results imply that the tasiARFs work together with mir1219 to increase auxin response in the tip cells of the growing protonema. Importantly, small RNA regulation is required for the stochastic expression of *PpARFb4*, suggesting that stochasticity is adaptive. As the authors suggest, stochasticity may contribute to developmental plasticity. Protonema with different capacities to develop into caulonema may allow the plant to respond effectively to changing environmental conditions. Indeed, the authors find that tasiARFs and mir1219 are required for the moss plant's response to low nitrogen levels. In wild-type plants, low nitrogen increases the formation of caulonema. This response is reduced in the *Ppsgs3* mutant and in plants expressing *PpARFb4-m*t*.*

To further explore the effects of small RNAs on the auxin GRN, the authors used a computational approach to model auxin susceptibility (or sensitivity) and network robustness. The complexity of the auxin GRN makes this a particularly challenging mathematical modeling project. However, several conclusions do emerge. First, both computational and experimental results show that tasiARFs increase auxin sensitivity in the protonema. Second, and consistent with other studies of small RNA regulation, tasiARFs increase robustness to intrinsic noise. Thus, variation in the expression of several auxin-regulated genes was substantially increased in the absence of small RNA regulation. The authors suggest that it is these qualities that have led to the frequent cooption of the *TAS3* pathway. This would explain why *TAS3*, together with clade B *ARF* genes, has been recruited to regulate so many different pathways.

Our future ability to mathematically model the entire auxin GRN will depend on the combination of experimental and computational approaches utilized by Plavskin et al. (2016). One important question that arises from this study is how stochasticity is conferred to protonema during development. Presumably, variable expression of *PpARFb* is conferred by finely tuned accumulation of tasiARFs. How this is accomplished is an important question for the future.

REFERENCES

Bennett, T. (2015). Trends Plant Sci. *20*, 498–507.

Erwin, D.H., and Davidson, E.H. (2009). Nat. Rev. Genet. *10*, 141–148.

Guilfoyle, T.J., and Hagen, G. (2007). Curr. Opin. Plant Biol. *10*, 453–460.

Plavskin, Y., and Timmermans, M.C. (2012). Cold Spring Harb. Symp. Quant. Biol. *77*, 221–233.

Plavskin, Y., Nagashima, A., Perroud, P.-F., Hasebe, M., Quatrano, R.S., Atwal, G.S., and Timmermans, M.C.P. (2016). Dev. Cell *36*, this issue, 276–289.

Prigge, M.J., and Bezanilla, M. (2010). Development *137*, 3535–3543.

Prigge, M.J., Lavy, M., Ashton, N.W., and Estelle, M. (2010). Curr. Biol. *20*, 1907–1912.

Wang, R., and Estelle, M. (2014). Curr. Opin. Plant Biol. *21*, 51–58.

Yamamuro, C., Zhu, J.K., and Yang, Z. (2016). Mol. Plant *9*, 57–70.

TBX5 and NuRD Divide the Heart

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In this issue of *Developmental Cell*, Waldron et al. (2016) identify an interaction between a master regulator of heart development, TBX5, and the NuRD complex and describe how mutations affecting the interaction may contribute to congenital heart disease. Furthermore, these interactions may have contributed to the evolution of cardiac septation.

The transcription factor TBX5 is a key regulator of heart development, with known roles in cardiac septation, conduction system development, and differentiation of cardiomyocytes (Mori and Bruneau, 2004). In humans, *TBX5* mutations are associated with Holt-Oram syndrome, an autosomal-dominant disease marked by hand and heart malformations, with the most common heart malformations being atrial septal defects (ASDs) and ventricular septal defects (VSDs) (Mori and Bruneau, 2004). Disease-associated mutations are predominantly within the DNA-binding T-box domain of TBX5, disturbing DNA binding and protein-protein interactions. For missense mutations outside the T-box, mechanistic insight is often lacking.

In this issue of *Developmental Cell*, Waldron et al. (2016) explore the in vivo TBX5